

Mortality of five wireworm species (Coleoptera: Elateridae), following topical application of clothianidin and chlorpyrifos

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ABSTRACT

Five wireworm species (*Agriotes obscurus*, *A. sputator*, *Limonius canus*, *Ctenicera destructor*, and *C. pruinina*) were exposed to clothianidin and chlorpyrifos at various concentrations using a Potter Spray Tower to compare larval susceptibilities to these compounds. Wireworms were stored in containers with soil at 15 °C after insecticide exposure, and their post-application health was evaluated weekly for up to 140 days. Where possible, LC₅₀, LC₉₀, LT₅₀, and LT₉₀ values were calculated and the LC₉₀ and LT₉₀ values of chemical concentrations compared between species. Considerable differences in susceptibility to both chlorpyrifos and clothianidin were observed among species, with the LC₉₀ of *L. canus* exposed to clothianidin being significantly higher than *A. obscurus* or *A. sputator*. Similarly, while the LC₅₀ of *A. sputator* exposed to chlorpyrifos was similar to that of *C. pruinina* and *A. obscurus* assayed in previous studies (0.05, 0.10, 0.10%, respectively), there was low (12.5%) mortality of *L. canus* at the highest concentration tested (0.15%). There were considerable differences in the survival of various wireworm species after exposure to clothianidin at 0.15%, with the LT₉₀ of *L. canus* (66.5 days) similar to those of *C. pruinina* and *C. destructor* (52.5, 59.5 days, respectively), but much shorter than those for *A. obscurus* or *A. sputator* (122.5, 115.5 days, respectively). Considerable differences in the induction of and recovery from morbidity induced by the chemicals were observed among species. Most larvae of *A. sputator* and *A. obscurus* exposed to chlorpyrifos were moribund before *C. pruinina* larvae (4, 7, 42 days after exposure, respectively). Most (proportion = 0.86) larvae of *L. canus* recovered from morbidity induced by chlorpyrifos, but a high proportion (>0.8) of moribund *A. sputator*, *A. obscurus*, and *C. pruinina* died. Larvae of *C. destructor* and *C. pruinina* which were moribund after exposure to clothianidin at 0.15% died or recovered sooner than larvae of *L. canus* and *A. obscurus*. Together these results suggest that the efficacy of both clothianidin and chlorpyrifos for wireworm control in the field are affected by the wireworm species present.

Key Words: *Agriotes obscurus*, *Limonius canus*, wireworm, contact toxicity, insecticide, survival time

INTRODUCTION

Wireworm problems are increasing across North America and Europe. In North America, the most important pest species include the Pacific Coast wireworm, *Limonius canus* LeConte, found from British Columbia (BC) to California (Horton and Landolt 2001), the dusky wireworm, *Agriotes obscurus* L. in BC, Washington and the Atlantic provinces (Eidt 1953, Vernon *et al.*

2001, Lagasa *et al.* 2006), the common click beetle, *A. sputator* L. in Atlantic Canada (Eidt 1953), and the prairie grain wireworm, *Ctenicera destructor* (Brown) in the Canadian prairies (Burrage 1963). A closely related species, the Great Basin wireworm, *C. pruinina* (Horn), is an increasing pest in the US Pacific Northwest (Kuhar *et al.* 2003). The increase in wire-

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worm problems, especially in Canada, is due at least in part to the loss of effective organochlorine (OC) and organophosphate (OP) insecticides, and the increased use of newer chemistries which are not as effective at reducing wireworm populations (van Herk *et al.* 2007, Vernon *et al.* 2007). Recent work has demonstrated that neonicotinoid (including thiamethoxam, clothianidin, acetamiprid, and imidacloprid), pyrethroid (e.g. tefluthrin) and spinosyn (i.e. spinosad) insecticides can cause long-term morbidity from which wireworms can eventually make a full recovery (van Herk *et al.* 2007, Vernon *et al.* 2007). In addition, tefluthrin, registered for wireworm control on corn in Canada, has been shown to be repellent to *A. obscurus* and *L. canus* in laboratory studies (van Herk and Vernon 2007b).

The efficacy of new insecticides for wireworm control is usually inferred from improvements in crop stand and marketable yield (van Herk and Vernon 2007b) and not from assessment of their direct effects on wireworms (exceptions are Hall and Cherry 1985, van Herk *et al.* 2007). This scarcity of toxicity data for wireworms is understandable, as subterranean insect larvae are often difficult to study *in situ*, and wireworms are difficult and costly to rear in the laboratory or collect from the field. Wireworm toxicity studies are complicated further by their recently discovered ability to make a full recovery after extensive periods of morbidity (van Herk and Vernon 2007a, Vernon *et al.* 2007). Wireworms exposed to sublethal doses of insecticide may appear dead and show no detectable movement to the unaided eye for up to 300 days (van Herk *et al.* 2007). Prematurely removing these "dead" wireworms from the study can easily lead to overestimations of an insecticide's effectiveness. Wireworms exposed to

other insecticides (e.g. fipronil) may not show symptoms of intoxication for several weeks before becoming moribund and dying (van Herk *et al.* 2007). Failure to conduct long-term observations of these wireworms can easily lead to underestimations of an insecticide's effectiveness. Although time consuming and expensive, laboratory bioassays in conjunction with field efficacy studies now appear to be requisite in developing a complete understanding of how candidate wireworm insecticides will work in practice.

Previous work has shown that the insecticide concentration required to kill 50% (LC₅₀) and 90% (LC₉₀) of *A. obscurus* are similar for clothianidin and chlorpyrifos, but the time required to kill 90% (LT₉₀) of larvae when exposed at near-LC₉₀ concentrations is much longer for clothianidin (123 days) than for chlorpyrifos (25 days) (van Herk *et al.* 2007). Preliminary work has also suggested that there may be differences in the toxicity of chlorpyrifos to *A. obscurus* and an additional species, *C. pruinina* (van Herk, unpublished data).

Bousquet (1991) lists some 369 known wireworm species in Canada, of which at least 30 are of economic importance (Glen *et al.* 1943; Wilkinson 1963). These species differ considerably in size and cuticle hardness (van Herk and Vernon 2007b). Thus the efficacy of various candidate insecticides for wireworm control may differ depending on the species present in the field. In this paper we present the LC₅₀, LC₉₀, and LT₉₀ values of clothianidin and/or chlorpyrifos topically applied to five wireworm species. The implications of differences in the relative toxicities and in the ability of these wireworms to recover from a moribund state are discussed.

MATERIALS AND METHODS

Wireworm collection and preconditioning. Five collections of wireworm larvae were made from different regions of North America. Late instar larvae of *C. pruinina* were collected in June 2004 from

an organic vegetable field near Boardman, Oregon (45°41'N, 119°50'W). Larvae were identified according to Glen (1950). Late instar *C. destructor* larvae were collected in July – August 2004 near Wainwright, Al-

berta (52°49'N, 110°52'W), and identified according to Glen et al. (1943). Larvae of *A. obscurus* were collected in March 2005 from a fallow field in Agassiz, BC, (49°14'N, 121°46'W) and identified according to Becker (1956). These larvae were at least 15 mm long when used in bioassays and thus three to four years old based on length criteria developed by Subklew (1934) for *A. obscurus*. Larvae of *L. canus* were collected in July 2005 from an organic vegetable farm in Kelowna, BC (49°49'N, 119°26'W), and identified according to Lanchester (1946). All *L. canus* were at least 14 mm long and therefore three to four years old (Wilkinson 1963). Late instar *A. sputator* larvae were collected in November 2005 near Kentville, Nova Scotia (45°06'N, 64°29'W), and identified according to Eidt (1953) and Becker (1956).

Larvae were stored, by species, at the Pacific Agri-Food Research Centre (PARC) in Agassiz, BC, in Rubbermaid® tubs (Newell Rubbermaid Inc, Atlanta, GA) filled with Agassiz soil at 15 – 20 °C until used. Agassiz soil (sandy-clay loam) was taken from a field at PARC, screened through 2 x 2 mm mesh to remove organic material, and dried to approximately 20% soil moisture by weight. Potato slices (cv. Russet Burbank) placed cut-face down on the soil provided food, as well as a means of selecting feeding wireworms for bioassays. Wireworms found feeding on potato slices were removed from the tubs, weighed, and placed in 150 ml plastic sample cups (Fisher Scientific Ltd, Ottawa, Ontario) filled with approximately 130 g Agassiz soil (for *A. obscurus*, *A. sputator*, and *L. canus*) or 170 g of a 2:1 mixture of Agassiz soil and clean sand (for *C. destructor* and *C. pruinina*). Five wireworms were placed in each cup no more than seven days prior to insecticide applications (see below).

A single piece (approximately 1 cm³) of peeled organic potato (cv. Russet Burbank), was placed in each wireworm storage cup. Lids were placed on cups after wireworms were inserted. Thereafter, wireworms were transported in Coleman® coolers (Sunbeam

Corporation (Canada) Ltd., Brampton, ON) to the Southern Crop Protection and Food Research Centre (SCPFRC) in London, ON. HOBO® H8 data loggers (Onset Computer Corporation, Pocasset, MA) placed inside the coolers indicated that the temperature remained between 8.5 and 20 °C during transport.

Insecticide application. Insecticides were applied directly to wireworms using a Potter Spray Tower (Burkhard Manufacturing Co Ltd, Rickmansworth, United Kingdom) at SCPFRC. Insecticides were dissolved in a 19:1 solution of acetone (histology grade, minimum 99% purity) and olive oil (Maestro® 100% Extra Virgin) (van Herk *et al.* 2007). Olive oil prevents the insecticides from coming out of solution and crystallizing on the insect cuticle, as sometimes occurs when insecticides are dissolved at high concentrations in pure acetone (van Herk, personal observation).

Just prior to insecticide applications, wireworms were removed from the cups and placed in an arena to check their health (see below). Healthy wireworms were placed in a 50 mm diameter x 4 mm deep sterile plastic Petri dish (Gelman Sciences, Ann Arbor, Michigan) in the tower, and are hereafter referred to as a single "batch" (four to five wireworms). Wireworms that were writhing were discarded. The shallow Petri dish used ensured that all wireworms in the batch received the same amount of spray. The tower was calibrated before the experiment to deliver 5.0 ml of insecticide solution in a uniform (11.9 cm diameter) application pattern (van Herk *et al.* 2007). Uniformity of spray deposition was visualized by applying 5.0 ml of a 0.01% (in acetone) red dye solution onto filter paper. This application indicated that the spray did not resolve into individual droplets, confirming that the olive oil did not interfere with the spray application.

For *L. canus*, *A. sputator*, and *A. obscurus*, eight to ten batches were exposed to each of five concentrations of clothianidin (0.005, 0.01, 0.05, 0.1, 0.15%) or chlorpyrifos (0.05, 0.075, 0.1, 0.125, 0.15%), or to the solvent alone. When one of these spe-

cies was selected for study, different batches were exposed to all concentrations of clothianidin or chlorpyrifos (plus solvent controls) on the same day. Due to the limited number of *C. pruinina* wireworms available, eight batches were exposed to clothianidin at 0.15% and eight batches to the control solution. Similarly, six batches of *C. destructor* were exposed to each of clothianidin at 0.15% and the control solution.

In a previous study, conducted in 2004, larvae of *C. pruinina* were exposed to chlorpyrifos (van Herk *et al.* 2007). Larvae of *C. pruinina* assayed in 2004 were collected at the same time, and preconditioned, selected and treated like *C. pruinina* assayed in 2006 (van Herk *et al.* 2007). Except for *C. pruinina* exposed to chlorpyrifos, all insecticide applications were conducted in January 2006.

Post-application observations. Treated wireworms were allowed to air-dry for approximately 1 minute, after which they were placed on the soil surface in their cups. Several minutes later, when the wireworms had burrowed into the soil, a fresh potato piece was placed in each cup, lids replaced, and the cups placed in a dark environmental chamber at 15 ± 0.2 °C. This temperature was selected to simulate the temperature of soil in spring, when pesticides would normally be applied in BC. Wireworms were inspected 1, 4, and 7 days after treatment (DAT) and every week thereafter for up to 140 days. After the first health check, done at SCPFRC, wireworms (except *C. pruinina* exposed to chlorpyrifos in 2004) were transported (as above) back to PARC where they were stored in growth chambers at 15 ± 0.2 °C. All subsequent health checks were conducted at PARC; health checks of *C. pruinina* exposed to chlorpyrifos in 2004 were conducted at SCPFRC (van Herk *et al.* 2007).

For each health check, wireworms were carefully removed from their cups with soft-touch forceps, and placed in the center of a 15 cm Petri dish lined with moistened filter paper (Whatman No.1, Whatman International Ltd., Maidstone, England).

Wireworm health was assessed according to Vernon *et al.* (2007), using the following criteria. Wireworms that could move out of a 10 cm circle drawn on the center of the filter paper within two minutes were designated as "Alive". Wireworms that were incapable of directed movement but capable of clearly visible movements were designated "Writhing". All wireworms that made no visible movements when gently prodded with forceps were inspected under a dissecting microscope and designated as "Leg & Mouthparts" if they were able to move their legs and mouthparts or "Mouthparts" if that was all they could move. Wireworms that were incapable of movement were considered to be dead. In all cases, wireworm death was confirmed by subsequent signs of decomposition which became visible within two weeks of death (van Herk, personal observation). Wireworms were removed from the study as soon as decomposition was evident; to ensure that morbidity did not recur, larvae that recovered from insecticide-induced morbidity were observed for two or more weeks after they had made a full recovery. Control wireworms were checked until the last insecticide-exposed wireworms of the species were removed from the study. Potato cubes were replaced each time wireworms were checked.

Statistical methods.

LC₅₀ and LC₉₀ analysis. The estimated concentrations required to kill 50% (LC₅₀) and 90% (LC₉₀) of larvae was computed, along with 95% confidence intervals, from the probit model (Southwood 1978, SAS Institute 2002). Due to the small number of wireworms per batch, the goodness of fit (GOF) of the probit model could not be computed from the standard chi-square distribution. *P*-values (with standard error (SE) estimates) were therefore computed using a parametric bootstrap procedure as described by van Herk *et al.* (2007). To accommodate data overdispersion, the variance of the binomial distribution was multiplied by a scale parameter (i.e. the deviance statistic computed for a concentration of a chemical divided by its degrees of freedom). Control wireworm mortality was

incorporated in LC analyses.

LT₅₀ and LT₉₀ analysis. Survivorship was modeled separately for each chemical with non-parametric Kaplan-Meier survival curves (Cox and Oakes 1984) using Proc LIFETEST (SAS Institute 2002). The time required for 50% (LT₅₀) and 90% (LT₉₀) of larvae susceptible to die at a certain concentration was estimated using these models. Standard errors were computed using a non-parametric bootstrap procedure as described by van Herk *et al.* (2007). The standard error of the LT values was then approximated by the standard deviation of the

bootstrap LT values. Parametric models with survival times following a Weibull distribution were tested, but provided a poor fit.

Comparisons. Pair-wise comparisons were made between various LC₅₀s and LC₉₀s by comparing the difference between two values to 0 with a Z-test. Tests were considered significant if $P \leq 0.05$. Comparisons between Kaplan-Meier curves were made using the log-rank test. Comparisons between individual LT₉₀ values were made with Wald tests.

RESULTS AND DISCUSSION

General observations. The wireworm species used in this study varied significantly in size, ranging from 13.7 mg (*A. sputator*) to 81.5 mg (*C. pruinina*) (Table 1). While some larvae were stored longer than others, the similar response of the same population of *A. obscurus* exposed to clothianidin in 2004 and 2006 (see below) suggested that storage did not affect wireworm susceptibility to insecticides.

Chlorpyrifos. The LC₅₀ and LC₉₀ of chlorpyrifos applied to *A. sputator* (Table 2) were similar to those previously calculated for *A. obscurus* (0.10, 0.14%, respectively; van Herk *et al.* 2007). Similarly, the LC₅₀ of chlorpyrifos applied to *A. sputator* was close to that calculated for *C. pruinina* (Table 2). However, there was low (12.5%) mortality of *L. canus* at the highest concentration tested (0.15%). Considering that *L. canus* is similar in size to *A. obscurus*, and much smaller than *C. pruinina* (Table 1), the lower susceptibility of *L. canus* to chlorpyrifos suggests that there may be differences in the efficacy of this chemical against different species when used in the field.

Considerable differences among species in the induction of and recovery from chlorpyrifos-induced morbidity were observed. All larvae of *A. sputator* and *A. obscurus* that died after exposure to chlorpyrifos at 0.10% were moribund (Writhing, Leg & Mouthparts, or Mouthparts) seven DAT,

but most *C. pruinina* that died showed no signs of morbidity until 42 DAT (Vernon *et al.* 2007). As wireworms can continue to feed on certain insecticides until they become moribund (van Herk *et al.* 2007), larvae that do not immediately become moribund during feeding may continue to damage crops. This suggests that for minimal wireworm damage and optimal population management, insecticides may need to be applied at concentrations that will induce morbidity quickly. A high proportion (>0.8) of moribund *A. sputator*, *A. obscurus*, and *C. pruinina* ultimately died (Vernon *et al.* 2007; data not shown for *A. sputator*), but most (12/14) moribund *L. canus* recovered, suggesting that morbidity alone is not always a reliable indicator of an insecticide's effectiveness.

Clothianidin. While the LC₅₀ of clothianidin applied to *A. obscurus* in 2006 was slightly lower than in 2004 (0.02, 0.07%, respectively; Table 2, van Herk *et al.* 2007), the LC₉₀ values were nearly identical (0.13, 0.15%, respectively; Table 2, van Herk *et al.* 2007), confirming previous results and justifying comparisons between the 2004 and 2006 studies. Similarly, the LC₅₀ and LC₉₀ values for *A. obscurus* (2006) were nearly identical to those for *A. sputator* (Table 2). In contrast, the LC₉₀ for *L. canus* was significantly higher than those for either *A. obscurus* or *A. sputator* ($P = 0.006$, $P = 0.019$, respectively), indicating

Table 1.
Mean (standard error) weight of wireworms used in toxicity studies.
Weight of *C. pruinina* includes wireworms exposed in 2004 study.

Species	<i>n</i>	Weight (mg)
<i>C. pruinina</i>	209	81.5 (2.31)
<i>C. destructor</i>	60	52.1 (2.72)
<i>L. canus</i>	440	21.4 (0.41)
<i>A. obscurus</i>	240	32.4 (0.59)
<i>A. sputator</i>	440	13.7 (0.25)

Table 2.
Toxicity of clothianidin and chlorpyrifos topically applied to various wireworm species in 2004 (*C. pruinina*) and 2006 (*L. canus*, *A. obscurus* and *A. sputator*). CL denotes 95% confidence limits.

Insecticide	Species	<i>n</i>	Slope (SE)	LC50 (CL)	LC90 (CL)	χ^2 (df)	<i>P</i> (SE)
clothianidin	<i>L. canus</i>	240	7.27 (1.77)	0.12 (0.08 – 0.16)	0.30 (0.18 – 0.41)	80.71 (54)	0.05 (0.007)
clothianidin	<i>A. obscurus</i>	240	12.25 (2.43)	0.02 (0.001 – 0.04)	0.13 (0.09 – 0.16)	85.54 (51)	0.009 (0.003)
clothianidin	<i>A. sputator</i>	240	9.92 (1.37)	0.02 (0.01 – 0.04)	0.15 (0.12 – 0.19)	29.04 (47)	0.998 (0.001)
chlorpyrifos	<i>C. pruinina</i>	130	6.58 (1.39)	0.10 (0.07 – 0.13)	0.30 (0.22 – 0.37)	13.81 (23)	0.907 (0.009)
chlorpyrifos	<i>A. sputator</i>	240	11.20 (1.65)	0.05 (0.04 – 0.07)	0.17 (0.14 – 0.20)	39.64 (48)	0.965 (0.006)

that the efficacy of this chemical in the field may also vary with species composition.

The LT_{90} of *A. obscurus* exposed to clothianidin at 0.15% in 2006 (Table 3) was similar to the LT_{90} of *A. obscurus* exposed to 0.1% and 0.25% in 2004 (143.5, 122.5 days, respectively; van Herk *et al.* 2007). The LT_{90} s of *A. obscurus* (2006) and *A. sputator* exposed to clothianidin at 0.15% were similar (Tables 3 and 4). While *L. canus* exposed to clothianidin at 0.15% died more quickly (66 days) than the two *Agriotes* spp., there was no significant difference in LT_{90} s between the species (Table 4). The difference in survival curves between *A. sputator* and *L. canus* (Table 4) reflects the faster initial rate of dying of *A. sputator* (Fig. 1). The LT_{90} s at 0.15% were significantly longer for both *Agriotes* species than for both *Ctenicera* species (Tables 3, 4). These results suggest that there are consid-

erable differences between wireworm genera in the time required to kill them after exposure to clothianidin, which may affect the effectiveness of the chemical when used for wireworm control.

While nearly all wireworm species were either moribund (writhing or appendage movement) 1 day after exposure to clothianidin at 0.15% 1 DAT (Fig. 1), differences in recovery from morbidity were observed. Initial recovery from the writhing or appendage movement stages took longer for larvae of *A. obscurus* and *L. canus* (56, 28 DAT, respectively) than for *C. pruinina* and *C. destructor* (4 DAT) (Fig. 1), suggesting that clothianidin applied at sublethal rates may be less effective in providing crop stand protection in fields infested with *C. pruinina* and *C. destructor*.

These laboratory assays demonstrate that the wireworm species tested differ in

Table 3.

Time (days) required for 90% mortality (LT90) for various wireworm species exposed dermally to clothianidin at 0.15%, as calculated from Kaplan-Meier survival curves. CL denotes 95% confidence limits.

Species	LT90 (CL)
<i>C. destructor</i>	59.5 (31.5 – 80.5)
<i>C. pruinina</i>	52.5 (45.0 – 66.5)
<i>L. canus</i>	66.5 (52.5 – 122.5)
<i>A. sputator</i>	115.5 (87.5 – 136.5)
<i>A. obscurus</i>	122.5 (66.5 – 136.5)

Table 4.

Comparison of LT90 values and Kaplan-Meier survival curves calculated for various wireworm species exposed dermally to clothianidin at 0.15%. LT90 values were compared with Wald tests; statistics shown are Z and P-values (respectively). Survival curves were compared with log-rank tests; statistics shown are Chi-square and P-values (respectively).

	<i>C. pruinina</i>	<i>L. canus</i>	<i>A. sputator</i>	<i>A. obscurus</i>
	LT90 values			
<i>C. destructor</i>	0.52, 0.60	0.23, 0.82	3.34, 0.0008	2.48, 0.013
<i>C. pruinina</i>	x	0.49, 0.62	4.80, < 0.0001	3.02, 0.003
<i>L. canus</i>	x	x	1.63, 0.10	1.57, 0.12
<i>A. sputator</i>	x	x	x	0.28, 0.78
	Kaplan – Meier survival curves			
<i>C. destructor</i>	0.70, 0.40	0.94, 0.33	12.11, 0.0005	4.70, 0.03
<i>C. pruinina</i>	x	2.39, 0.12	23.13, <0.0001	10.28, 0.0013
<i>L. canus</i>	x	x	6.70, 0.009	2.36, 0.12
<i>A. sputator</i>	x	x	x	1.29, 0.26

the onset and recovery of morbidity and the occurrence of mortality following exposure to certain insecticides (chlorpyrifos and clothianidin). Since several wireworm species are known to attack many agricultural

crops worldwide, field efficacy trials should be carried out on as many economic species as possible to establish application rates that will provide crop damage and/or wireworm population control for all species.

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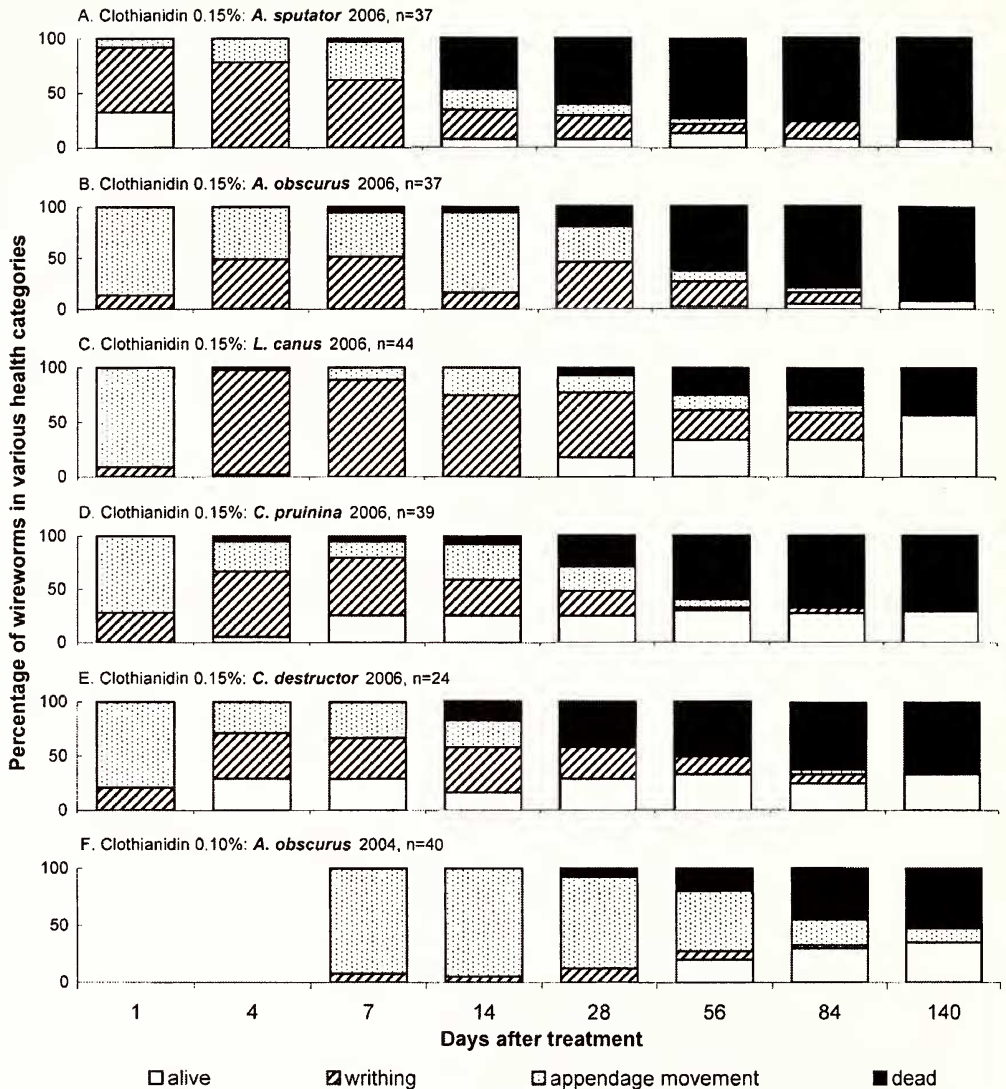


Figure 1. Transitional stages of toxicity in *A. sputator*, *A. obscurus*, *L. canus*, *C. pruinina*, and *C. destructor* wireworms exposed dermally to clothianidin in a Potter Spray Tower in 2006, and in *A. obscurus* wireworms exposed to clothianidin in 2004. The percentage of wireworms Alive, Writhing, with Leg and/or Mouthpart Movement (Appendage Movement), or Dead are shown on various dates of observation. Data for *C. pruinina* exposed to clothianidin 0.15% first appeared in Vernon *et al.* 2007.

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